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2918

Accumulation of dye in Nitella as related to dissociation.

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In previous papers[1] the writer has described the accumulation of brilliant cresyl blue in the sap of *Nitella* and has discussed certain theoretical explanations. The present paper deals with the possibility of explaining accumulation on the basis of dissociation as recently discussed by Osterhout.[2] For this purpose we may assume that the dye enters in the form of dye hydrate (DOH) but that ions are unable to pass in or out. At equilibrium DOH has the same concentration inside the cell as in the outside solution, but if the pH value is lower in the sap there will be more dissociation, and consequently the total concentration of the dye (ions plus undissociated molecules) will be greater than in the outside solution. The difference in concentration can be calculated if the dissociation constant of the dye and the pH values inside and outside are known. When the previous papers were published, such calculations were attempted but at that time the experiments were not sufficiently complete for this purpose. Since then the writer has been able to secure the necessary data.

Living cells of *Nitella* were placed at 25 ± 0.5° C. in 0.00002 M dye solutions at different pH values, from pH 6.4 to pH 9.2 (M/150 phosphates or borates). At definite intervals the cells

[1] Irwin, M., *J. Gen. Physiol.*, 1925-26, viii, 147.

[2] Osterhout, W. J. V., *J. Gen. Physiol.*, 1925-26, viii, 131.

were removed from the solutions. The end[3] of each cell was then cut and the sap was squeezed out upon a glass slide. The sap was then drawn up into a capillary tube, the color of which was matched with that of the capillary tube containing the standard dye solution.

It was found that the greater the pH value of the outside solution, the higher was the rate of penetration, and the greater the concentration of the dye in the cell sap at equilibrium. A maximum was reached at pH 9, where further increase in pH value of the external dye solution brought about no increase in the rate of accumulation.

At pH 6.4, 6.6, and 6.9 the process reached an equilibrium, but at higher pH values the cells died before the equilibrium was attained. When the rates taken at a very early stage of the time curve are plotted as ordinates, with the pH values of the external dye solutions as abscissæ, an S-shaped curve is obtained which resembles the curve obtained when the amount of dye absorbed by chloroform is plotted as ordinates against the pH values as abscissæ. In both cases the curve reaches a maximum at about pH 9, beyond which further increase in the pH value brings about no increase in the amount of dye taken up by chloroform or in the rate of penetration into *Nitella*. This is regarded as indicating that the percentage of undissociated molecules has reached its maximum value. This maximum is therefor taken as 100 per cent in each case and the amount of DOH at different pH values is calculated on this basis. When these values are plotted against pH values, the curves agree, though at lower pH values the curve obtained with *Nitella* is a little below the curve for chloroform. This discrepancy may be due either to experimental error or to conditions in the cell. The theoretical curve calculated from the dissociation constant of the dye agrees fairly well with these two curves. This may indicate that the rate of accumulation is dependent on the concentration of DOH in the external solution.

If we assume that equilibrium is reached when DOH inside is equal to DOH outside, the total concentration of dye at equilibrium will depend on the concentration of DOH in the outside solution and on the degree of dissociation of the dye in the sap (*i. e.*, on the concentration of D^+ ions). Calculations made upon this basis agree well with the observed facts.

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[3] For details of technique, see Irwin, M., *J. Gen. Physiol.*, 1925-26, viii, 147.

It may be added that it is possible, by making certain assumptions, as in the case of CO_2 discussed by Osterhout and Dorcas[4] to account for the relation between the inside and outside concentrations of dye on the basis of Donnan equilibrium. But in that case we might expect the rate of penetration to increase as the percent of dissociation increases, which is contrary to observation.

The high temperature coefficient (about 4.8) indicates that the dye does not pass in by simple diffusion but combines chemically with some constituent of the protoplasm on its way into the vacuole: or if it passes in by simple diffusion the process is complicated by other factors.

It is evident that the accumulation of dye may be explained on the basis of dissociation as set forth in the present paper or on other grounds as described in previous papers. Which of these explanations is correct may remain for the present an open question.

2919

Successive changes in the electrocardiogram following acute coronary artery occlusion.

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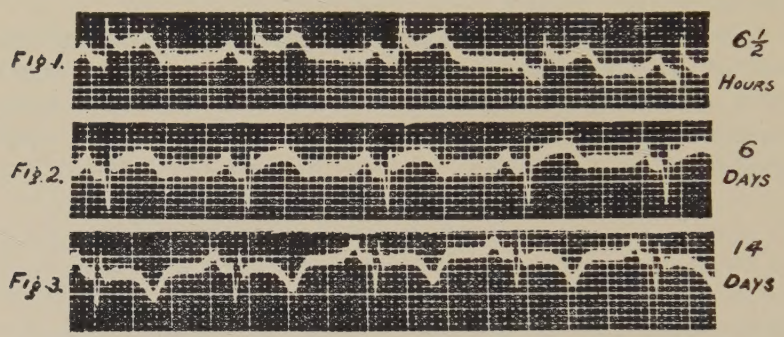
By the use of the portable electrocardiograph[1] it has been possible to secure a series of electrocardiograms in four patients soon after the onset of symptoms diagnostic of coronary artery occlusion. As none of these patients died, there was no opportunity to confirm the diagnosis by a pathological examination of the coronary arteries, but the diagnosis is justified by considerable experience in similar cases in which one or two characteristic electrocardiograms were obtained, which also presented typical symptoms and associated phenomena, and in which necropsy con-

[4] Osterhout, W. J. V., and Dorcas, M. J., *J. Gen. Physiol.*, 1925-26, ix, 255.

[1] Mann, H., PROC. SOC. EXP. BIOL. AND MED., 1925, xxiii, 19.

firmed the diagnosis of acute coronary artery closure. The subsequent course in these four patients was also consistent with the original diagnosis.

In general, the electrocardiographic changes may be divided into two stages. The first stage, which was seen as early as 6½ hours after the onset of original pain, consists of a well-defined deviation from the normal electrocardiogram, namely, the R-T transition, is abnormally elevated above the base line. In other words, after the completion of the R wave, the curve fails to return to the base line until the completion of the T wave. This is illustrated in Fig. 1. Indications of the electrocardiographic



Electrocardiograms taken shortly after acute coronary occlusion. The upper electrocardiogram shows lead 2 as recorded 6½ hours after the acute onset of symptoms. This curve exhibits the characteristics of the first stage. The middle electrocardiogram shows lead 2, six days after the onset. The lower curve shows lead 2 fourteen days after the onset. This curve shows the characteristic features of the second stage.

second stage appeared as early as 32 hours after the onset of clinical symptoms, but the change was not fully developed until the lapse of at least 12 days in one instance, and of 2 or more weeks in the others. The second stage (illustrated in Fig. 3) consists of a change in the T wave. The T wave is more definitely separated from R, due to the fact that the R-T transition approaches the base line; the T wave may be inverted, and assumes a characteristic form; *i. e.*, the first limb is curved, the apex peaked and the second limb is rather straight. This we have called a "coveplane" T. Pardee[2] has described one case in which the electrocardiograms presented these two stages. After an interval of

[2] Pardee, H. E. B., *Arch. Int. Med.*, 1920, xxvi, 244.

6-8 weeks from the onset of the attack, the T wave may lose its characteristic shape and approach the normal. From our experience in other cases of coronary artery occlusion, we know that the cove-plane T may persist for years. On the other hand, we have seen no example of long duration of the changes of the first stage, *i. e.*, marked elevation of the R-T transition. The general similarity of the successive changes in the four cases cannot be regarded as accidental. Therefore, it may be said that the early electrocardiographic changes associated with acute coronary artery occlusion are first seen in the R-T interval, and then in the T wave itself.

2920

Lactic acid of normal and pathological spinal fluids.

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In 1917 Levinson[1] observed that spinal fluids from cases of epidemic meningitis immediately after withdrawal had a subnormal pH. This was in some instances further decreased when the fluids were permitted to stand at room temperature unstoppered. However, normal and tuberculous meningitic fluids when freshly drawn showed similar figures for pH, and the pH increased on standing, particularly in the tuberculous fluids. Levinson believed that the lowering of the pH of the spinal fluid in meningococcus meningitis was due to the accumulation of lactic acid in the fluid, but he presented no data to substantiate this explanation.

In the present communication, data are presented for the sugar and lactic acid of the spinal fluid of normals, miscellaneous pathological conditions and meningitis. Clausen's method was employed for the determination of the lactic acid, and the Folin-Wu procedure for the sugar.

Specimens of spinal fluid obtained from five normal adults, after a night's fast and rest, showed lactic acid concentration from 8 to 15 mg. per 100 cc. In twenty-one miscellaneous non-menin-

[1] Levinson, A., *J. Infect. Dis.*, 1917, xxi, 556.

gitic cases the lactic acid varied from 9 to 26 mg. per 100 cc. Fluids obtained from two chronic nephritics after convulsions gave figures of 22 to 23 mg., and from two cases of *encephalitis lethargica* 26 and 23 mg. Clinical improvement in the instances of encephalitis was accompanied by a fall in the spinal fluid lactic acid to 18 and 17 mg. respectively. Meningismus associated with broncho-pneumonia in three children gave normal figures for the spinal fluid lactic acid.

Meningococcus meningitis in the seven patients studied presented figures for the spinal fluid lactic acid varying from 23 to 77 mg. per 100 cc. In all instances there was observed a decrease in the sugar, and in some no sugar was found. The amount of lactic acid produced did not account for all the sugar lost. Similar changes in the spinal fluid sugar and lactic acid were noted in influenza and pneumococcus meningitis. In one case the intraspinal administration of anti-meningococcus serum resulted in a continuous fall in the lactic acid from 30 to 7 mg. within 10 days time. This patient recovered. However, in another instance the lactic acid continuously rose from 37 to 77 mg. although the serum was given intraspinally within 3 days, and the patient died.

The lactic acid of ten tuberculous meningitic fluids varied from 11 to 33 mg. Figures exceeding 20 mg. were obtained in those subjects suffering from an active pulmonary or miliary tuberculosis with meningitis. The intraspinal administration of anti-meningococcus serum to cases of tuberculous meningitis produced an increase in the spinal fluid lactic acid which appeared to run parallel with the increase in the cells.

A comparison of sugar and lactic acid of the spinal fluid and of the whole, venous blood was made in two normals and thirteen pathological cases. The specimens were obtained during fasting and resting state and as nearly at the same time as possible. The lactic acid content of normal blood varies from 11 to 15 mg. per 100 cc. The sugar of the spinal fluid was found to be from 60 to 70 per cent of that of the blood, except in two cases of meningitis. Here the spinal fluids gave no reaction for sugar, although both specimens of blood showed a hyperglycemia. The lactic acid, however, in the spinal fluids had a concentration from 80 to 90 per cent of that of the blood. In two instances of epilepsy, specimens of blood and spinal fluid were obtained about 20 minutes after convulsions. The lactic acid of both the blood and spinal fluid was increased more than 100 per cent above that of

the control specimens from the same patient, and in these specimens taken after convulsion the concentration of the lactic acid in the spinal fluids was greater than that of the blood.

2921

The effects of repeated intravenous injections of India ink on the blood picture in rabbits.

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The following investigation was undertaken in order to study the effects of the repeated intravenous injection of India ink upon the blood picture in rabbits.

Higgins' waterproof India ink was used. It was filtered and suspended in physiological salt solution in the proportion of one part of ink to three parts of salt solution. The suspensions were sterilized before each injection. With few exceptions, ten cubic centimeters of the ink suspension were injected into the ear veins of the rabbits, at intervals of forty-eight hours. The number of injections varied between seventeen and fifty-six. The rabbits weighed between two and one-half and three kilos.

The following constituents of the blood were studied: erythrocytes, hemoglobin, leucocytes and reticulated cells. Examinations were made immediately preceding injections. Similar examinations were made on a series of normal control rabbits kept under the same conditions.

In all the rabbits, as a result of the injections, there was a gradual fall in the number of erythrocytes, which at the lowest levels was forty to fifty per cent of the original count. Following this drop, in spite of continued injections, there was a rise in the erythrocyte count which reached approximately normal values at about the twentieth injection.

Determinations of hemoglobin were made with the Newcomer apparatus, using daylight as the source of illumination. The values obtained with this apparatus are only relative, but they tended to follow the fluctuations in erythrocyte counts except in

one rabbit, in which the fluctuations were less pronounced than those of the other three rabbits.

Normoblasts were usually observed in every smear. The normoblasts became more numerous as the number of injections increased and hemacytoblasts appeared.

The reticulated cells were moderately increased during the period of the anemia, but the count remained within normal limits during the period of recovery. This is contrary to the usual findings in experimental anemias, in which there is a marked increase in the number of reticulated cells during the recovery period.

There was a moderate leucocytosis associated with the early injections. Subsequently the leucocyte count remained within the limits of normal variations. The differential counts showed no marked changes.

The structural changes in the organs associated with the repeated ink injections will be described in detail in a later publication.

2922

Bacterial flora of nose and throat in health and upper respiratory infection.

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In searching for the causative agent in respiratory disease, the problem is seriously complicated by the fact that the nose and throat normally harbor a variety of bacteria, some constantly present and others more or less transient. Correct interpretation, therefore, of the importance of organisms found in respiratory disease is dependent upon comprehensive familiarity with the bacterial flora in health. In the present study, a preliminary

* This study was made possible by a grant from the Chemical Foundation Company.

in an investigation of the Common Cold being undertaken by us, observations have been made of the bacterial flora of the nose and throat in a group of normals over a comparatively long period, and qualitative and quantitative changes occurring in the course of upper respiratory infections appearing in the group have been noted.

Thirteen individuals were studied for periods ranging from five to nine months. Aerobic and anaerobic cultures were made from the nose and throat infections weekly in health, and daily in the course of colds and throat infections. All organisms present were carefully identified and their prominence noted.

Our results may be summarized briefly as follows: The normal basic nasal flora includes *staphylococcus albus,* diphtheroids, and for certain individuals, *staphylococcus aureus* and *citreus;* occasional transients are gram-negative cocci and non-hemolytic streptococci. The normal basic throat flora includes gram-negative cocci, non-hemolytic streptococci, and for certain individuals large gram-positive cocci, *B. pfeifferi,* both non-hemolytic and hemolytic, and diphtheroids; transients are *staphylococcus albus, staphylococcus aureus,* hemolytic sterptococci, *staphylococcus citreus* and pneumococci.

Certain of these organisms have been assumed to play pathogenic roles. Such so-called potential pathogens are hemolytic streptocci, *staphylococcus aureus,* pneumococci, and *B. pfeifferi.* In the nose, hemolytic streptococci were found once without associated untoward symptoms. In the throat, hemolytic streptococci had a high incidence in four cases, of whom two had no associated symptoms and two had more or less continuous subacute inflammation of their throats. *Staphylococcus aureus* was high in one case and prominent in her colds. Pneumococci were associated with a mild sore throat in one case and no symptoms in another. High incidence of *B. pfeifferi,* both hemolytic and non-hemolytic, was not accompanied by any apparent increase in respiratory infection.

In the course of colds a number of changes from the normal were noted. In the nose there was a tendency toward scantiness of growth in the early cultures and the basic flora was decreased; in the throat the usual prominence of gram-negative cocci was less marked and there was a moderate increase in the prominence of non-hemolytic streptococci. In both nose and throat there was an increase in the incidence of the so-called potential pathogens.

In the nose hemolytic streptococci and *B. pfeifferi* showed increases which were due entirely to late secondary spread. In the throat, *staphylococcus aureus,* hemolytic streptococci, and *B. pfeifferi* went up; in the case of the two former this was due to secondary spread. The increase of *B. pfeifferi* was due mainly to the fact that the organism was widespread, in normals as well, at the time of most of our colds (late winter and spring). It also played an important part as a late secondary invader.

No bacteria were found in the first or in early cultures to which an etiological role could be attributed. The indications are that the so-called pathogens noted above probably play a secondary role in the type of upper respiratory infection under investigation.

Cultures taken during sore throats showed the expected increase, in the throat, of hemolytic streptococci (from 17 per cent in normals to 56 per cent during infection).

2923

Glutose and its biochemical behavior.

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Glutose, a 3 ketohexose, prepared by the regulated action of alkali on glucose or fructose, has been investigated.

Chemically it resembles glucose in several ways. On treatment with sodium hydroxide it is converted into hydroxy acids, chiefly optically inactive lactic. On warming with phenylhydrazine in weakly alkaline solution it yields the osazone of methyl glyoxal. Zinc ammonium hydroxide converts it into methyliminazole.

Biologically it differs from glucose in several ways. In human beings, when taken by mouth, it is almost quantitatively eliminated, about half in the urine and half by bowel. Human diabetics show no change in glucose excretion when fed glutose, and the glutose is eliminated as in normals. Phlorhizinized dogs excrete glutose given subcutaneously quantitatively in the urine, and no extra glucose is formed. The respiratory quotient of 0.77

in two normals was not changed after glutose. Glutose did not protect rabbits from insulin shock. Of four individuals on high fat diet, three showed no reduction of ketosis after glutose, while one did. Thus most of the evidence points toward glutose being inert in the body.

It is of great interest in this connection that yeast did not form a hexosephosphate with glutose under conditions that led to ready hexosephosphate formation with glucose. *B. coli* was found to yield both acid and gas on glutose broth.

Methods of preparation and estimation will be described in a subsequent communication.

2924

The ionic nature of amylase.

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By studying the distribution of trypsin and pepsin between suspended particles of gelatin and the fluid surrounding them, Northrop[1] has been able to show that these enzymes behave like univalent ions, the former being a cation and the latter an anion. We have carried out some preliminary experiments in which Northrop's procedure has been used to investigate the ionic nature of amylase.

Particles of iso-electric gelatin were suspended in solutions, the pH of which was varied by the addition of HCl or NaOH. On the alkaline side of the iso-electric point a small amount of KCl was added to furnish an ion (Cl^-) which could easily be titrated. The suspensions were stirred constantly for 2 hours. The solution containing the enzyme[2] was then added, and stirring was

[1] Northrop, J. H., *J. Gen. Physiol.*, 1924, vi, 337; *Ibid.*, 1925, vii, 603.

[2] "Taka-Diastase" (Parke-Davis) was used in these experiments. In a few instances a 5 per cent aqueous solution was used. It was found more convenient, however, to employ a 20 per cent solution of this preparation in 60 per cent alcohol.

TABLE I.

pH	Cl- gel. / Cl- filtrate	Amylase (gel.) / Amylase (filtrate)	Trypsin (gel.) / Trypsin (filtrate)
7.2	1/3.3	1/5	1.7
7.2	1/2.0	1/11	
7.2	1/3.3	1/5.1	
7.1	1/2.1	1/14	
6.4	1/2.8	1/17	1.6
5.6	1/2.0	1/6	
5.5	1.3	1/1.3	
4.3	1.45	15.	
4.3	1.35	14.	
4.1	5.6	1.2	
3.9	3.5	41.	1/3.9
3.9	2.6	19.	1/2.3
3.8	1.3	7.5	
3.8	4.5	1.2	
3.5	4.4	3.4	
3.5	4.4	3.3	1/5.1
3.35	6.1	1.1	1/2.8

continued for 2 hours more; the temperature was maintained between 0° and 4° C. The gelatin was then separated from the surrounding fluid by filtration, and the concentration of amylase in the filtrate and in the melted gelatin was determined by a viscometric method.[3]

In the accompanying table the ratios of the amylase concentration in the gel to that in the filtrate at various hydrogen ion concentrations are compared with the corresponding ratios of the Cl^- ions[4] in the 2 phases. Since the preparation used contained a small amount of trypsin, the ratios for this enzyme were also determined in a few instances.[5] The figures in the table represent single experiments.

As can be seen from the table, the distribution of amylase between the gelatin and the filtrate is extremely variable from pH 3.8 to 5.6. It would seem that forces other than those of the Donnan Equilibrium play a part here. This is in harmony with the observations of Northrop on pepsin that adsorption occurs near the iso-electric point.

A certain regularity is, however, found in the ratios obtained farther from the iso-electric point. On the acid side, the concentration of amylase is always greater in the gel, while on the alkaline side more amylase is constantly found in the filtrate. Although the data are not sufficient to warrant conclusions as to the valence of amylase, the distribution seems to indicate that this enzyme, like pepsin, is a negatively charged ion.

The few determinations of the trypsin ratios are in agreement with Northrop's observations on this enzyme.

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[3] Davison, W. C., *Johns Hopkins Hosp. Bull.*, 1925, xxxvii, 281.

[4] Chlorine ions were determined by titration in the filtrate; the Cl^- concentration in the gel being calculated by difference.

[5] Trypsin determinations were made by the method of Northrop and Hussey, *J. Gen. Physiol.*, 1923, v, 353.

2925

Experimental study of action of hyoscine hydrobromide on development of the nervous system of amblystoma.

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The success attendant upon the treatment of post-encephalitis by hyoscine hydrobromide at various clinics, has given rise to considerable speculation as to just how the drug acts. Various suggestions will be found in the literature; the majority of which suggest the action of the drug on the basal ganglia. The careful studies of Coghill[1] on the development of the nervous system of *Amblystoma punctatum* have given us an excellent tool for studying the effect of drugs on the nervous system. By subjecting growing embryos to a solution of the drug it is possible to determine, with a considerable degree of accuracy, the portion of the nervous system upon which the drug acts. The experimental evidence here reported shows that the site of action of the drug can be quite sharply delimited. Young embryos of *Amblystoma* in the premotile stage were placed in solution of hyoscine hydrobromide in concentrations varying from 1:3,000, 1:5000, and 1:10,000. A selected series of embryos was also placed in tap water as controls. At frequent intervals, varying in length from two hours to twelve hours, the embryos were stimulated gently with a single hair. The normal progression in the development of the reflexes, as shown by Coghill,[2] occurred in both the controls and hyoscinized embryos up to the stage of the development of the swimming movement. At this time, the hyoscinized embryos either failed to respond by the usual swimming movement or gave only a flick of the tail. The controls, on the other hand, developed the swimming movements normally. Coghill's researches have correlated the functional stages in motor response to stimulation with anatomical findings in the development of the nervous system. The failure of the response of the hyoscinized embryos by swimming movements, and their

[1] Coghill, G. E., *J. Comp. Neur.*, 1924, xxxvii, 37.

[2] Coghill, G. E., *J. Comp. Neur.*, 1924, xxxvii, 37.

almost perfect response to early reflexes, indicates beyond a doubt that the action of the drug is not on the neuro muscular mechanism, nor in the spinal cord reflex pathways, nor in the association centers in the medulla, but rather its action is on the integrative mechanism cephalad to the medulla, which controls the swimming movements. The evidence, then, points to the action of the drug upon the basal ganglia.

2926

Penetration into valonia of oxidation-reduction indicators; estimation of the reduction-potential of the sap.

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The penetration of 2,- 6,- dibromo phenol indophenol into *Valonia* was observed when the external pH was varied from 5 to 9, and when the internal pH was changed from normal (6.4) to 5.2 and to 9.0. It was found that when the pH of the sap was normal, the penetration of the dye into the sap follows the course of a bimolecular reaction curve, and the amount of dye in the sap at equilibrium is proportional to the amount of undissociated dye in the external solution. When the sap is made more acid than normal, there is more dye present; when the sap is more alkaline there is less dye present. At higher temperature and lower concentrations, the curves follow a course like that for two consecutice unimolecular reactions. 2,- 6,- dibromo phenol indophenol was found in the sap only in a completely reduced form. Its concentration was estimated colorimetrically after it had been reoxidized *in vitro*.

Methylene blue was found to penetrate into the sap in an oxidized form. This dye is a very strong base and is completely dissociated at all pH values used in these experiments. The amount found in the sap did not vary with external nor with internal changes in pH from 5 to 9.

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K-indigo tetrasulphonate was found to penetrate into the sap in a yellow form.

K-indigo disulphonate could not be found in the sap by means of the method used.

The electrode potential or E_h of the protoplasm was tentatively found to be between .21 and .48 subject to certain assumptions. The E_h of the sap was found to be between .12 and .15, subject to certain assumptions. By further calculations, the rH or the logarithm of the reciprocal of the hydrogen pressure of the sap was found to be between 16 and 18; that of the protoplasm was less exactly defined.

In this connection it is interesting to make a comparison with the work of Needham and Needham[1] who found the rH of the cell interior of *Amoeba proteus* to be between 17 and 19 (subject also to certain assumptions). It is quite striking that the rH value for the sap of *Valonia* and that for the cell-interior of *Amoeba proteus* should be so nearly alike since they are such widely separated forms and since the methods of experimentation were so different. It may be that this value, when much more accurate methods of experimentation are used, is the same for all forms of life, or that there is a slightly lower oxidation potential in animals than in plants as seen from Needham's work.

2927

A method for the experimental production of lung abscess.

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(Introduced by Peyton Rous).

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The study of lung abscess in the laboratory has been handicapped by the difficulty of reproducing such a lesion in the experimental animal. It occurred to us that this might be due to the fact that almost all investigators started with the assumption that lung abscess was caused by the aspiration of infected material,

[1] Needham, J., and Needham, D. M., *Proc. Roy. Soc.*, B, xcviii, 259.

and, therefore, devoted their energies towards the production of such lesions by the placement of infected materials in the trachea and bronchi. Recent studies in other types of postoperative lung complications would seem to indicate that emboli from the operative field were the dominant factor in the ensuing complication. We felt that lung abscess might well have a similar etiology.

Method.

The method consists in liberating into the venous blood stream infected emboli which ultimately lodge in the lung. The animal is anesthetized with ether, following the administration of morphia, 1/6 or 1/4 gr. The right jugular vein is exposed and isolated for about three centimeters. A transverse incision is made large enough to admit a glass cannula about 0.8 cm. in diameter. Bleeding is controlled by bull-dog clamps, or by tapes passed around the vein and weighted with clamps. An embolus of infected tissue,* is then placed in one end of the glass cannula, which is introduced into the jugular vein. The other end is connected with a large syringe containing salt solution and the embolus is forced into the circulation by emptying the syringe. The opening in the vein is closed with silk.

In reviewing the results obtained so far, we find that in twelve out of seventeen attempts we were able to produce a definite lung abscess by the introduction of an infected embolus into the jugular vein. (Figs. I to IX.)

In fifteen instances the embolus lodged in the left lower lobe, and in only two instances in the right lower lobe. This is explained on the basis of the more direct and straight course pursued by the left pulmonary artery as compared to the right pulmonary artery in the dog, and by the larger and more direct channel that characterizes the artery to the left lower lobe as compared to the other branches of the left pulmonary artery.

* The embolus is prepared as follows: The femoral vein is isolated, its branches are ligated, and a segment 12 to 18 millimeters long is removed. One end of this segment is ligated with silk. The other end is held open by three silk sutures. Into the lumen of this small segment of vein, an emulsion of bacteria is introduced by a platinum loop, together with one or two small bits of lead, previously coated with paraffin so as to render them inert. A drop of blood is then added and the second end tied. The small bits of metal are added in order to study roentgenologically the final resting place of the infected embolus.

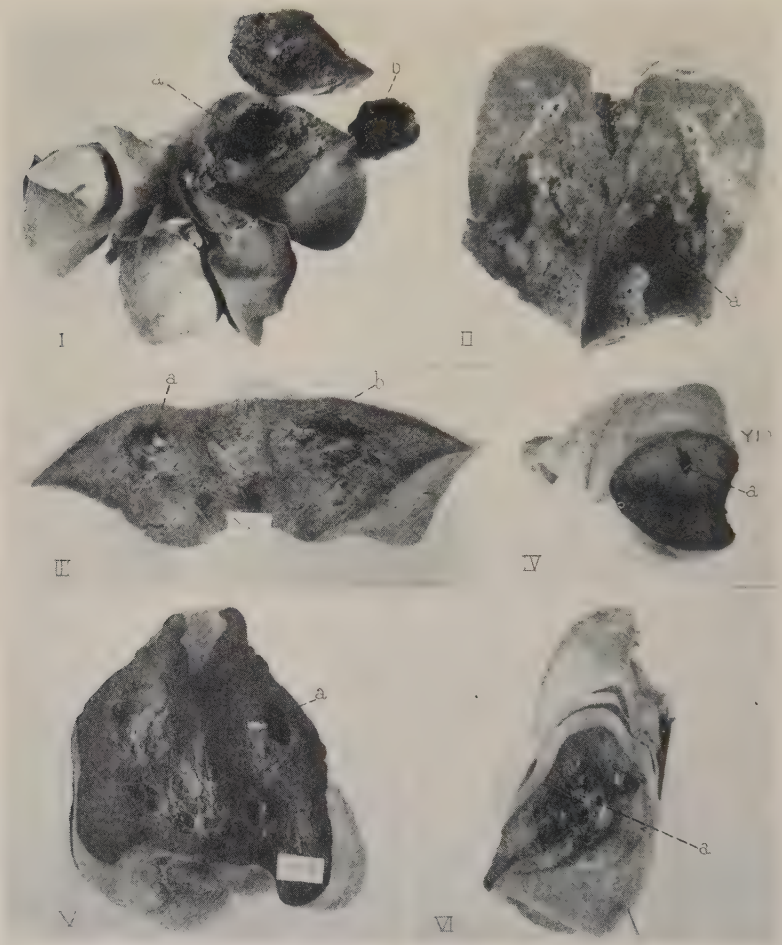

FIG. I. Lung removed from Dog Y 5, 5 days after introduction of infected tonsil tissue into left jugular vein.

a—abscess cavity.

b—blood clot removed from cavity.

FIG. II. Left lower lobe removed from Dog Y 16, 8 days after introduction of infected tonsil tissue into jugular vein.

a—large abscess which ruptured into pleural cavity.

FIG. III. Left lower lobe removed from Dog Y 20, 6 days after introduction of segment of vein impregnated with *staphylococcus aureus* and *B. coli* into jugular vein. The animal is still living.

a—lead filing in abscess cavity.

b—segment of vein in abscess cavity.

FIG. IV. Left lower lobe removed from Dog Y 10, 15 days after introduc-

tion into jugular vein of muscle tissue impregnated with *staphylococcus aureus.* The animal is still living.

a—lead filing in abscess cavity.

FIG. V. Left lower lobe removed from Dog Y 18, 10 days after introduction of segment of vessel impregnated with *staphylococcus aureus* and *B. coli.* The animal is still living.

a—lead filing in abscess cavity.

FIG. VI. Left lower lobe removed from Dog Y 13, 14 days after introduction of small cylinder of potato impregnated with *staphylococcus aureus* into jugular vein.

a—potato fragment in abscess cavity.

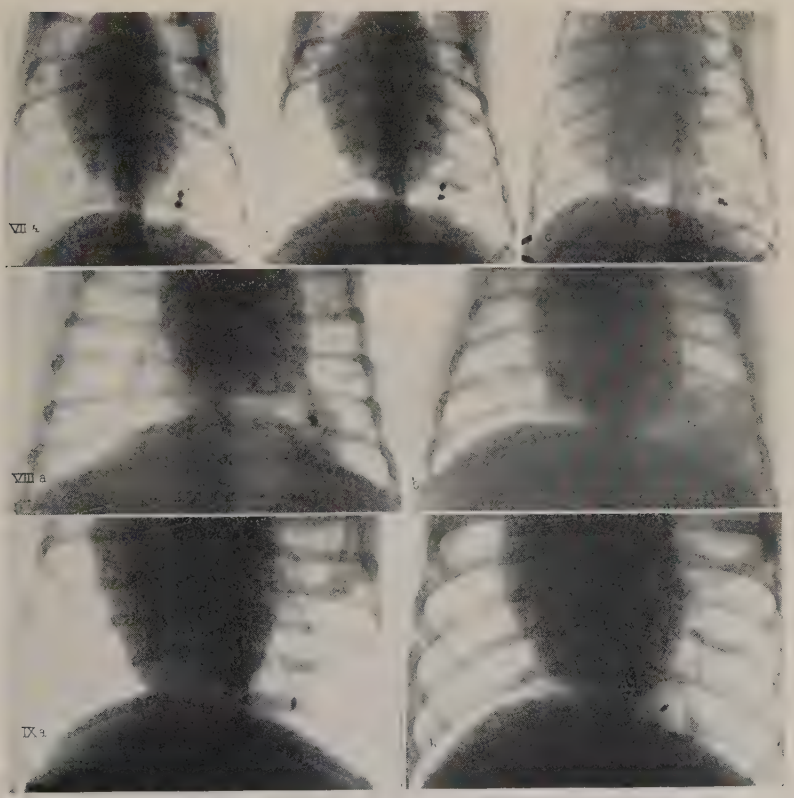

FIG. VII. Roentgenograms of Y 10 following introduction of infected embolus into jugular vein.

a—on the third day.

b—on the fifth day.

c—on the seventh day.

FIG. VIII. Roentgenograms of Y 20 following introduction of infected embolus into jugular vein.

a—on the second day.

b—on the sixth day; marked cavitation.

FIG. IX. Roentgenograms of Y 19 after introduction of infected embolus into the jugular vein.

a—on the second day.

b—on the ninth day.

In five instances an abscess failed to develop. At necropsy, two of these cases presented the signs of a healing infarct with scarring and pleural adhesions on the surface of the affected lobes. In three animals, the roentgenograms showed no evidence of abscess formation, and the dogs have remained well.

Conclusions.

Thus far our experiments have led us to believe that we can produce lung abscess with a fairly high degree of success and the method may perhaps furnish further experimental evidence in favor of the embolic theory of lung abscess.

2928

A continuous electrical method of recording the volume-flow of blood.

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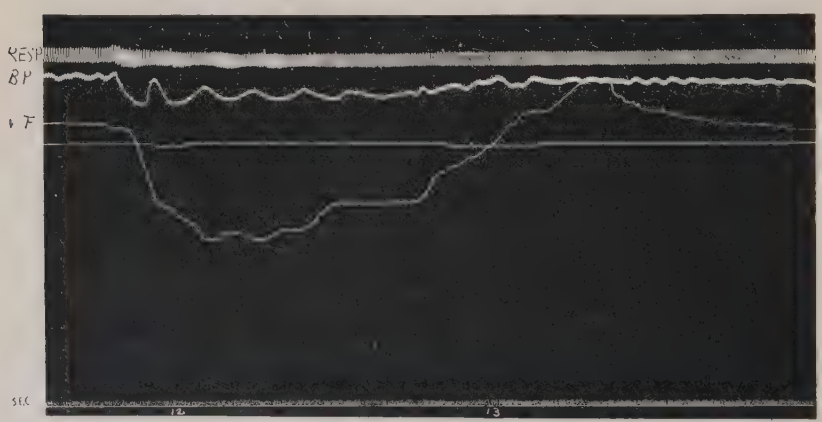
The method employed is based on the principle that the amount of heat, radiated by circulating blood led through a tube, varies



with the volume-flow. This heat is measured by means of a thermopile placed in the course of a stream of water exposed to the radiation of heat from the blood. The volume-flow (E. M. F.) is recorded potentiometrically on smoked paper with the method described by Gesell and Hertzman.[1]

The accompanying figure shows the effects of hemorrhage (50 cc.) on the volume-flow of blood through the carotid artery of a dog, followed by reinjection of the blood plus 10 cc. of 6 per cent dextrose solution.

2929

The effect of digitalis upon the refractory period of the ventricular muscle.

FRANK N. WILSON, GEORGE R. HERRMANN and SHELBY W. WISHART.

[*From the Heart Station, University of Michigan Hospital, and the Department of Medicine, Tulane University, New Orleans, La.*]

By a method similar to that used by Lewis and Drury we have tested the effect of digitalis upon the refractory period of the ventricular muscle of the dog. Lewis and Drury found that strophanthin increased the refractory period of the auricular muscle. We were somewhat surprised when we were unable to demonstrate that digitalis had a similar effect upon the refractory period of the ventricle, the more so, because digitalis has been shown by Cohn and Jameson to produce striking changes in the *T*-wave of the electrocardiogram.

The drug was given intravenously. After control tests of the refractory period were made, one and five-tenths to two cubic centimeters of the tincture diluted ten times with physiological saline solution were given. Ten to fifteen minutes later the refractory period was redetermined. A second dose was then given and this procedure was repeated until the experiment was ended by ventricular fibrillation, which usually occurred upon stimula-

[1] Gesell, Robert, and Hertzman, Alrick B., PROC. SOC. EXP. BIOL. AND MED., 1925, xxii, 298.

tion after three to four doses. In no experiment did a conspicuous increase in the refractory period occur.

Since the length of the refractory period is greatly influenced by the heart rate, it was, of course, necessary while the tests were being made to maintain artificially a constant rate considerably above the natural level. Between tests the heart was allowed to beat naturally. It was found that when the heart rate was suddenly raised, the refractory period did not at once drop to the level that it afterwards reached and maintained. In our first experiments the influence of the previous rate of beating upon the determination made immediately after a change in heart rate, was not realized. Confusing variations in the refractory period were, therefore, encountered.

In these experiments the possibility of variation in the threshold of excitability was eliminated by determining the value of the threshold stimulus from time to time.

2930

Effect of pilocarpin upon the cardiac mechanism in circus rhythm with ventricular tachycardia.

FRANK N. WILSON, SHELBY W. WISHART and GEORGE R. HERRMANN.

[*From the Heart Station, University of Michigan Hospital, and the Department of Medicine, Tulane University.*]

Pilocarpine is known to stimulate the cardiac fibers of the vagus. The site of its action is in the neighborhood of the myoneural junction. Since vagus stimulation tends to increase the rate of the circus rhythm in auricular flutter and fibrillation and also to reduce the ventricular rate, pilocarpin might be expected to produce these effects.

We have given pilocarpin hydrochloride intravenously, in doses of one-sixteenth to one-eighth grain, to a number of patients with auricular fibrillation or flutter. In the majority of cases no definite effect was produced either upon the ventricular rate or upon the auricular mechanism. In one instance short attacks of auricular flutter were immediately abolished. In two cases of extreme ventricular tachycardia which followed the administration

of quinidin, pilocarpin produced a sudden and abrupt fall of ventricular rate. In one of these cases, in which the auricles were fluttering, this was associated with a slight rise in the rate of the circus rhythm.

The action of pilocarpin, upon the vagus, in the dosage referred to, is very feeble. Nevertheless, the drug may occasionally prove useful in the suppression of post-quinidin tachycardia. The tachycardia which follows the administration of quinidin in cases of auricular fibrillation and auricular flutter is due to a depression of the rate of the circus rhythm, combined with partial vagus paralysis. It may be extreme and may cause the patient serious discomfort. The action of pilocarpin is peripheral to that of quinidin, and although its action is feeble it may increase vagal tone sufficiently to greatly reduce the ventricular rate.

2931

Nature of abnormal ventricular complexes during quinidin treatment of auricular fibrillation.

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[*From the Heart Station, University of Michigan Hospital, and the Department of Medicine, Tulane University.*]

When quinidin is given to patients with auricular fibrillation there is almost invariably a considerable increase in the ventricular rate. This is accompanied in one-third to one-half of the cases by the appearance of groups of abnormal ventricular complexes. These abnormal complexes must be due either to abnormal impulse formation in the ventricular muscle or to defective intraventricular conduction. The former explanation has been advanced by Cohn,[1] Levy,[2] and Lewis[3]; the latter by White,[4] and others.

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[1] Cohn, A. E., Personal Communication.

[2] Levy, *Arch. Int. Med.*, 1922, xxx, 474.

[3] Lewis, T., Drury, A. N., Wedd, A. M., and Iliescu, C. C., *Heart*, 1922, ix, 254.

[4] White, P. D., Marvin, H. M., and Burwell, C. S., *Boston M. and S. J.*, 1921, clxxxv, 647.

We have studied two cases in which these abnormal ventricular complexes were unquestionably due to defective intraventricular conduction. In the first case, runs of abnormal ventricular complexes occurred after eighteen grains of quinidin. After twenty-four grains all of the ventricular complexes were of the abnormal type. The administration of one-eighth grain of pilocarpin, intravenously, slowed the ventricular rate, and the longer pauses were followed by relatively normal complexes. There was, however, no close relation between the length of the pause and the form of the complex which followed. About two hours later, the auricular fibrillation gave place to normal rhythm but the ventricular complexes were still of the abnormal type. The normal type of complex returned, however, as soon as the quinidin effect wore off. After the rhythm had been normal for 3 days, eighteen grains of quinidin failed to produce abnormal complexes. The patient was then given three grains of quinidin twice a day to prevent a return of fibrillation but without success. When fibrillation returned the ventricular rate was rapid and runs of abnormal complexes again occurred. These disappeared when quinidin was discontinued and could not be produced by the intravenous administration of one-fiftieth grain atropin, which greatly increased the ventricular rate.

In a second patient quinidin converted auricular fibrillation into auricular flutter, and runs of abnormal complexes bearing a definite and constant relation to the flutter waves occurred. A similar relation between the flutter waves and the abnormal complexes produced by quinidin may be seen in a curve published by Levy.[5]

We have given single twelve grain doses of quinidin to twenty patients with heart disease, but without arrhythmia. Several other patients of the same type have received larger doses. In none of these patients did either single ventricular extrasystoles or runs of ventricular extrasystoles occur. In many instances there was a slight increase in the ventricular rate; in four instances inverted T waves became less inverted or upright.

We attribute the majority of the abnormal ventricular complexes, produced by quinidin in auricular fibrillation, to a combination of two factors: depression of intraventricular conductivity, and, accelaration of the ventricular rate. We do not deny

[5] *Archiv. Int. Med.*, 1922, xxx, 474, Fig. 7.

that the drug may occasionally induce abnormal impulse formation in the ventricular muscle, but satisfactory proof that it does so is as yet wanting.

2932

Changes in the electrocardiogram following the arsphenamine treatment of cardiac and aortic syphilis.

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[*From the Heart Station, University of Michigan Hospital, and the Department of Medicine, Tulane University.*]

We have recently observed conspicuous changes in the electrocardiogram following the administration of arsphenamine in four cases of cardiac and aortic syphilis.

A patient with syphilitic myocarditis and complete right bundle branch block developed an abnormal idioventricular rhythm following the administration of two-tenths gram of arsphenamine and died a few days later. For several weeks preceding the treatment his condition had been stationary.

Two patients with syphilitic aortitis, but without definite signs of cardiac syphilis, and with practically normal electrocardiograms, developed diphasic complexes suggesting incomplete bundle branch block, following intensive arsphenamine therapy. In one of these patients the T-wave changes gradually disappeared but the QRS changes persisted. The other patient could not be followed. Similar but less conspicuous changes occurred in a third patient with syphilitic aortitis who showed great enlargement of the heart and left ventricular preponderance before treatment.

These observations indicate that the administration of aspenamine in cases of cardiac syphilis may sometimes be followed by myocardial changes of an undesirable kind. The slow development and persistence of the electrocardiographic changes suggest that they are not due to a local Herxheimer reaction, although this possibility cannot be excluded. The rapid destruction of the luetic lesions and their replacement by scar tissue with

injury to the intraventricular conducting system, directly or through interference with the coronary circulation, is a possible cause.

Similar treatment produced no changes in the electrocardiograms of five other patients with aortic syphilis. In twenty patients with primary and secondary lues intensive arsphenamine treatment produced no electrocardiographic abnormalities. A study of about sixty patients with primary and secondary lues has convinced us that reliable clinical or electrocardiographic evidence of involvement of the heart or aorta during this stage of the disease is decidedly rare.

2933

Factors influencing distribution of potential differences, produced by heart-beat, at surface of body.

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[*From the Heart Station, University of Michigan Hospital, and the Department of Medicine, Tulane University.*]

In order to understand the influence of the position of the electrocardiographic electrodes upon the form of the electrocardiogram it is necessary to have some idea of the laws which govern the flow of electric currents in solid conductors.

When a constant difference of potential is produced in a thin conducting sheet of infinite extent, the potential (V_1) of any point of that sheet is determined according to the following equation: $v_1 = \frac{Q}{2\pi dk} \log_e \frac{r_1}{r_2}$ in which Q is the quantity of electricity flowing in unit time, d the thickness of the sheet, k the conductivity of the material of which the sheet is composed, and r_1 and r_2 the distances of the point from the sink and the source respectively. In dealing with the difference in potential between two points at a given instant the expression $\frac{Q}{2\pi dk}$ may be replaced by k. Then

[1] Electrokinetics, *Ency. Brit.*, 11th Ed., ix, 216.

$$v_1 - v_2 = k\left(\log_e \frac{r_1}{r_2} - \log_e \frac{r_3}{r_4}\right)$$

in which r_3 and r_4 are the distances of the second point from the sink and source respectively. In the case of a solid conductor a similar expression holds:[2]

$$v_1 - v_2 = k_1\left[\left(\frac{1}{r_1} - \frac{1}{r_2}\right) - \left(\frac{1}{r_3} - \frac{1}{r_4}\right)\right]$$

in which the letters r_1, r_2, r_3, and r_4 have the same significance as before.

The body is not a conductor of infinite extent, nor can it be assumed that all body tissues have the same conductivity. The potential differences produced by the heart are not constant. Nevertheless, the potential differences produced by the heart-beat within the body or at its surface are determined by factors similar to those which appear in the equations given above.

Within the heart during the period of its electric activity a great number of sinks and sources exist. It may be demonstrated theoretically and experimentally that the differences of potential produced by the heart-beat at the body surface are of much greater magnitude in the immediate neighborhood of the heart than at a distance from it. If one electrode be placed over the heart, the position of the second electrode, so long as it be placed at a distance from the heart, has comparatively little effect upon the form of the ventricular electrocardiogram. Under these circumstances the curve obtained resembles those obtained in animals by placing one electrode upon the exposed heart and the other upon the chest wall. When one electrode is placed nearer the heart than the other, that part of the heart which lies nearest this electrode exerts a preponderating effect upon the form of the resulting curve. Leads of this type are semi-direct leads. The principles underlying Einthoven's equilateral triangle do not apply when the electrodes are placed near the heart.

Other factors remaining unchanged, an increase in the conductivity of the body tissues as a whole or an increase in the conductivity of the heart muscle or of tissues lying near the heart will decrease the magnitude of potential differences at the body surface. This may explain the small amplitude of the electrocardiogram in certain patients with edema, ascites, hydrothorax, or pericardial effusion.

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[2] Pierce, B. O., *Newtonian Potential Function*, Ginn & Co., 3rd Ed., 248.

2934

Globoid bodies and their occurrence in cultures.

WILLIAM H. HARRIS.

[*From the Department of Pathology, Tulane University, New Orleans, La.*]

The term "globoid bodies" was first used by Flexner and Noguchi[1] in 1913 as a descriptive term for the minute spherical microorganisms cultivated by them from the filterable virus of poliomyelitis. Bashford and Wilson[2] likewise cultivated "globoid bodies" from lethargic encephalitis. Duval and I[3] have recently described a "globoid microorganism" in cultures of the blood from Dengue fever.

The "globoid bodies" described by Flexner and Noguchi consist of minute spheres or "coccoids" measuring from 0.15 to 0.3 microns in diameter and arranged in pairs, short chains, and masses. These bodies are best demonstrated tinctorially by the Giemsa method and are readily seen in dark-field preparations. Cultivation of the "globoids" has been obtained in the Smith-Noguchi tissue media. Growth has also been successful by the employment of Noguchi's Ringer-plasma medium devised for the cultivation of the *Leptospira*. The culture of the poliomyelitis globoids appears as an opalescent haze about the tissue, increasing for a period of five days, after which there occurs a gradual sedimentation. In our cultures of Dengue Globoids, either in the Smith-Noguchi media or Noguchi plain plasma mixture, visible growth was manifested by minute clear cut spherical or oval colony formation. These colonies were seen only after 2 or 3 weeks incubation, were densest at the bottom of the culture tube, gradually appearing smaller and more sparsely scattered as the upper portion of the medium was approached. No growth was visible for the upper inch just beneath the capping layer of paraffin oil. The colonies are comparatively larger below, measuring, however, only approximately 0.5 mm. and fading into pin-point size at the upper limit of the growth. No growth has been noted upon the surface of the medium. It is evident, therefore, that

[1] Flexner, Simon, and Noguchi, N., *J. Exp. Med.*, 1913, xviii, 461.

[2] Bradford, Bashford, and Wilson, *Brit. Med. J.*, 1919, i, 599.

[3] Duval and Harris, *J. Exp. Med.*, 1924, xl, 835.

globoids are anaerobic in their cutural nature. After several generations we were unable to perpetuate growth of the Dengue Globoids.

The various cultures of globoids have occasioned, in the lower animal, the infection similar to that from which they had been previously obtained.

There are at present nearly fifty etiological agents designated as filterable viruses that are pathogenic for man and lower animals. For some of these viruses, microorganisms which are either easily seen, such as the leptospira, or seen with difficulty, as the "globoid bodies," have been brought to view either through culture on special media or through employment of the dark-field. Very recently in the work of Barnard and Gye[4] upon cancer, the use of the special ultra violet ray microscope has visualized what they consider to be a very minute organism even of a smaller type than the "globoid bodies." In this connection the Twort-D'Herelle bacteriophagum may also be mentioned as an almost intangible entity; however its animate nature is questioned.

"Globoid bodies" are very minute forms which are regarded by some as truly animate entities and by others as artefacts formed particularly in special protein media. Thus Twort and Twort,[5] in work upon the etiology of influenza, undertook the cultivation of an ultramicroscopic agent in this disease. They obtained colonies along the stab puncture which were later believed by them to be crystalization processes of salts in solution put in action by some small point of particulate matter acting as a nucleus. Laidlow[6] obtained somewhat similar colonies to Twort and Twort. He found that when these "cultures" were boiled for 5 minutes or autoclaved for 30 minutes that subplants yielded similar pseudo colonziation, thus apparently demonstrating their inanimate nature. The examination of these pseudo colonies, when cut out from the media, showed under the microscope sphero-crystals in masses. The chemical examination demonstrated that these masses consist of crystallization of calcium and magnesium salts of fatty acids, and were thus really calcium and magnesium soaps. Laidlow believes that these soaps may remain dispersed in a complex colloidal system such as found in certain media, but that inoculation may furnish a focus of crystallization upon which

[4] Barnard and Gye, *Lancet*, London, 1925, ii, 117.

[5] Twort, F. W., and Twort, D. N., *J. of Hyg.*, 1921, xx, 85.

[6] Laidlow, P. P., *Brit. J. Exp. Path.*, 1925, vi, 36.

crystals will accumulate and form a nucleus of a pseudo-micro-organismal colony. It is noteworthy that these authors do not describe any morphology suggestive of microorganisms in the smear preparation of these pseudo-colonies. They also state that this crystallization deposit can be kept indefinitely from subplant to subplant. On the other hand, the globoid bodies obtained by us in cultures from Dengue fever, exhibit a definite microscopic morphology for the units comprising the colonies. Unlike the crystalline pseudo-colonies, their growth could not be indefinitely perpetuated. In this connection Rosenow[7] has stated that certain aerobic bacteria, such as streptococcus, may grow anaerobically as very minute forms. However, they will assume their normal basic morphology when planted upon suitable media under aerobic conditions. The Dengue globoids grow better in the deeper areas where anaerobiasis is more marked and they do not colonize in the upper level or on the surface where aerobes would grow more prolifically. Neither did growth occur on subplants under favorable aerobic conditions.

The inoculation into guinea pigs of cultures containing these globoidal microorganisms, obtained from the blood of patients infected with Dengue Fever, reproduced the febrile and leucocytic reactions present in this disease.

There exists certain artefacts which resemble the colonization observed for globoid bodies, but these are inanimate structures and can be differentiated from the living microorganismal forms.

We believe globoid bodies are minute living microorganisms of a much smaller size than the usual very small recognized forms. They have a definite structure consistent with true microorganisms. They can be grown in culture which when inoculated into the experimental animal reproduce the disease from which they were primarily isolated.

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[7] Rosenow, *J. Inf. Dis.*, 1918, xxii, 281.

2935

The chemical nature of insulin.

CASIMIR FUNK.

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For the past two years we have suspected that the tendency to look upon insulin as a simple chemical compound might be erroneous, and that the diminution of the unit dose does not mean a purification in the strict sense, but rather a splitting off of some inactive amino acids. This conclusion was reached from the extremely small content of chlorine in the insulin hydrochloride. Some time ago we had a second proof that our contention is right. In studying the chemical composition of the insulin compound obtained with naphtol yellow S, or its sodium salt, it has been found that fairly pure preparations of insulin, containing about 10 clinical units to a milligram (impure insulin gave unsatisfactory results), give a precipitate with naphtol yellow S, the filtrates being absolutely inactive. The insulin compound is insoluble in water and glacial acetic acid, but soluble in dilute acetic acid and soda solution. It can be injected into rabbits with typical insulin action in the form of the sodium salt, which does not become inactive even on standing in alkaline solution. The activity is destroyed by trypsin. Judging from the content of naphtol yellow S in the compound we can imply that the molecular weight of insulin is about 700. On analysis, the ratio of nitrogen to sulfur was found to be 11:1. This means that in free insulin it would be N:S = 20:1. It seems, at present, that there is but one sulfur atom in the insulin molecule. The chemical nature of insulin is perhaps that of a complicated polypeptide. The data dealing with the recovery of free insulin from the compound, and full analytical results, will appear shortly.

Some insulin preparations were supplied to us by the British Drug Houses, Ltd., London, through the kindness of Dr. H. H. Dale.

Western New York Branch

Clifton Springs Sanitarium, December 12, 1925.

2936

The biological value of cereal proteins in human nutrition.

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Two different studies, one upon five human subjects, the other upon four, were carried on with a view to discover whether amongst the respective proteins of corn, wheat and oats, as presented to the American public in the form of cereal breakfast foods, any difference in biological value could be detected. In the first study the method of Karl Thomas[1] was followed and the so-called "metabolic nitrogen" determined in control periods of two days each. Alternating with these periods were periods of three days, during each of which the cereals successively supplied nitrogen in amount equal to the total nitrogen excreted in the control periods, or nearly so. The chief objections to the Thomas method were the tendency to diarrhoea during the control periods when the theoretical energy supply was derived solely from cornstarch, heavy cream and sugar, and the difficulty of making such a diet palatable. There was no material difference amongst the three cereals as regards biological value whichever method of calculation was employed.

In the second study control periods of three days each alternated with cereal periods of four days each; but instead of a protein-free diet, a milk-cream-fruit diet was used deriving 80 per cent of the nitrogen from milk, 10 per cent from heavy cream and 10 per cent from fruit. The tendency to diarrhoea was negligible and the diet was palatable. In the cereal periods the milk

[1] *Archiv. f. Physiologie*, 1909, p. 219.

protein was replaced by cereal protein, fruit and cream remaining the same, the total calories being equalized by variations in the amount of sugar and corn starch taken. Wheat products gave slightly better replacement values than oats and corn products.

2937

Vitamine studies with menhaden fish meal and menhaden oil.

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Experiments were carried out with white rats in which the value of menhaden fish meal and menhaden oil was tested as a preventive or cure of Xeropthalmia, using various basal rations. Without exception the results were as follows:

1. Fish meal fed at levels of 10, 20 and 30 per cent did not prevent Xeropthalmia, nor cure it.
2. Menhaden oil fed at levels from 2 to 30 mg. daily both prevented and cured Xeropthalmia.
3. An alcoholic extract of fish meal fed at levels which made the fat content of the basal ration equal to that of the above mentioned menhaden oil rations, did not cure nor prevent Xeropthalmia.

The fish meal used in these studies was a product that had been on hand for some time. In showing that as small an amount as 2 mg. daily of menhaden oil prevented Xeropthalmia, it became evident that originally the fish meal must have contained vitamin A, since considerable of the oil is left in the residue after pressing. However, since neither the high percentage of fish meal fed, nor the concentrated alcoholic extract was effective, it must be concluded that the vitamin remaining in meal after pressing is destroyed in later processing or in storage.

Further studies were carried out to observe the influence of fish meal and menhaden oil on calcification. For these studies the fish meal and menhaden oil were obtained directly from the manufacturer, the menhaden oil having been expressed from the sample of fish meal in question.

Steenbock's rachitic ration consisting of yellow corn 76, wheat gluten 20, calcium carbonate 3, and sodium chloride 1, was used. Forty-eight rats were divided into four groups as follows: group 1, basal ration; group 2, basal ration plus 2 mg. menhaden oil daily; group 3, basal ration plus 2 mg. cod liver oil daily; group 4, basal ration in which 20 per cent of fish meal was substituted. At the end of 1, 2 and 3 weeks four rats were removed from each group and the ash content of the femur and tibia determined—a measure of calcification suggested by Dutcher.[1] The results were as follows:

AVERAGE ASH CONTENT OF FEMURS.

Ration	No. of weeks		
	1	2	3
Basal	43.86 ± .61	39.42 ± .89	40.19 ± .31
Basal + 2 mg. menhaden oil	44.29 ± .76	36.67 ± .39	37.50 ± .41
Basal + 2 mg. cod liver oil	39.83 ± .61	45.45 ± .28	44.99 ± .76
Basal + 20 per cent fish meal	49.79 ± .84	52.08 ± .71	57.14 ± .26

Over the three-week's period the ash content decreased with the basal ration and was increased by the addition of cod liver oil, as was expected. The addition of the 2 mg. of menhaden oil had little influence in increasing the ash content over that of the basal ration. Thus the amount of the oil which proved adequate for curing Xeropthalmia had no apparent influence on calcification.

Because of the large percentage of fish meal used in the fourth ration the calcium and phosphorus relations were made somewhat more favorable for calcification than was the case with the basal ration, and the increased calcification obtained with the fish meal ration is not proof that the meal contains the antirachitic factor. Further studies of this question are in progress.

[1] Dutcher, R. A., *Penn. An. Rpt.*, 1925.

2938

Specific dynamic action and muscular efficiency on exclusive cereal and meat diets.

O. H. GAEBLER and CHARLES A. MORRISON. (Introduced by J. R. Murlin).

[*From the Department of Vital Economics, University of Rochester, Rochester, N. Y.*]

Measurements of the energy metabolism were made by means of the Benedict "Universal" respiration machine and of nitrogen excretion in the urine. Calculations were made by the Zuntz and Shumburg method. Work was performed on a bicycle ergometer fitted with a Prony brake. Dynamic action was obtained on alternate days in a reclining position. The schedule of rest and work periods was so arranged that the results of the work experiments could be compared directly with the specific dynamic action of the last meal. The meat used was practically free of fat. A wheat cereal and an oats cereal in the form of breakfast foods cooked equal lengths of time were used in two parallel series. Three subjects ate these foods in iso-caloric amounts.

The results showed that the higher dynamic action of meat did not produce less efficiency in amounts of work up to 16,000 kgm. per hour. The wheat cereal gave slightly higher dynamic effects and slightly higher net efficiency than the oats cereal, owing to more rapid digestion and absorption. Changes in carbon dioxide equilibrium and the recovery period after work were compensated.

2939

The rate of glycogen formation in the liver during glucose absorption.

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An attempt has been made to obtain data for a graphic representation of the normal rate of glycogen formation in the liver of the rat. If such a curve could be constructed with the desired degree of accuracy, a standard of comparison for even small changes in the rate of glycogen formation would be available.

Experimental.

Male rats were used, that were between two and three months of age and weighed between 120 and 150 grams. They were fasted for 48 hours in order to reduce the glycogen content of the liver. In one series glucose alone was given, in a second series 15 units of insulin per 100 gm. of body weight were injected simultaneously with the sugar feeding. The amount of sugar absorbed was determined on each rat.[1] The experiments were made at a temperature of 24° C. ± 2°.

Seven rats, fasted for 48 hours, showed an average of 0.397 per cent liver glycogen. This amount of glycogen was subtracted in each case in order to obtain the amount of glycogen formed.

The curve illustrating the normal rate of glycogen formation is S — shaped. The rate of glycogen formation increases gradually up to the second hour. Between the second and the third hour the rate is at its maximum. Between the third and fourth hour the rate diminishes until quite suddenly a definite glycogen maximum is reached after 4 hours. The curve for the insulinized rats shows how profoundly the rate of glycogen formation is influenced by a large dose of insulin. With a smaller dose of insulin the curve would be steeper, with a still larger insulin dose than was used in our experiments the curve would be still flatter.

Discussion.

When sugar is absorbed from the intestine, it passes through

[1] For the method used see the following report.

TABLE I.

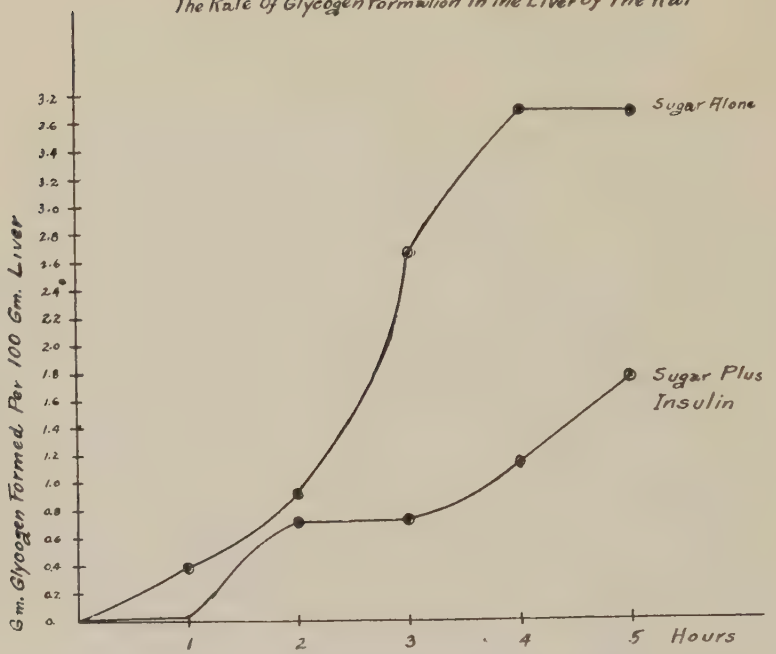
The rate of glycogen formation in the rat liver during the absorption of glucose from the intestine. A. after giving glucose alone. B. after giving glucose plus insulin. Each figure is an average of 5 to 6 experiments.

A. Glucose alone.					B. Glucose plus insulin.					
Liver in per cent of body weight.	Absorption coefficient.*	Blood sugar.	Glycogen formed per 100 gm. liver.	Percentage of sugar absorbed that is built up into glycogen.	Liver in per cent of body weight.	Absorption coefficient.*	Blood sugar.	Glycogen formed per 100 gm. liver.	Percentage of sugar absorbed that is built up into glycogen.	Remarks.
	gm.	mg.	gm.	per cent		gm.	mg.	gm.	per cent	Glucose absorption
2.96	0.183	169	0.38	6.07	3.00	0.169	73	0	0	1 hour
2.88	0.188	201	0.91	7.19	3.09	0.180	68	0.71	5.85	2 hours
3.13	0.176	189	2.66	15.84	2.89	0.175	60	0.73	3.73	3 hours
3.22	0.176	178	3.68	18.46	3.06	0.169	70	1.14	5.39	4 hours
3.24	0.175	173	3.65	13.70	3.20	0.189	71	1.77	6.01	5 hours

*The absorption coefficient is the amount of sugar absorbed per 100 gm. of body weight per hour.

Figure 1

The Rate Of Glycogen Formation In The Liver Of The Rat



the liver before it comes in contact with the other tissues of the body. In previous work on glycogen formation the amount of sugar passing through the liver was an unknown factor. Nor was it posible to know whether the control animals and the injected animals absorbed the same amount of sugar. Furthermore, most authors confined themselves to the determination of one point out of the whole curve of glycogen formation. Since different authors chose different points of the curve, certain discrepancies in the results were bound to occur. The advantage that is gained when rats instead of large laboratory animals are used is obvious. Rats can easily be bred in sufficient numbers so that animals of uniform stock, age and nutritional condition can be used for the experiments.

Our experiments show that the insulinized rats, which absorbed the same amount of sugar as the controls, deposited decidedly less liver glycogen. Lesser[2] found that mice, which re-

[2] Lesser, E. J., *Die innere Sekretion des Pancreas,* in *Oppenheimer's Handbuch der Biochemie,* 1924, 2d ed., vol. ix, 159.

ceived glucose and insulin intraperitoneally, formed glycogen 3 times as fast as the controls. Our experiments do not contradict his results. The important factor is the relation of the sugar content of the cells to the amount of insulin present. In Lesser's experiments, where sugar was injected intraperitoneally, an excess of sugar entered the blood and the tissues in a short time.[3] Consequently, the sugar content of the tissues relative to the insulin dose was great. In our experiments, where sugar was absorbed from the intestine, the relation was reversed. The normal rat, with the aid of its own insulin production, is able to metabolize the sugar at the same rate at which it enters the blood from the intestine. In other words, the relation of insulin to the amount of sugar available is optimal with respect to the rate of glycogen formation. Therefore, the injection of even a small insulin dose will create an excess of insulin over the amount of sugar available. On 8 rats, not recorded in Table I, 1 unit of insulin instead of 15 was injected, with the effect that within two to three hours less glycogen was deposited than in the respective controls.

Summary.

1. A curve for the normal rate of glycogen formation in the liver of rats during glucose absorption has been constructed.

2. Large doses of insulin decrease the rate of glycogen formation in the liver very markedly.

[3] The rate of absorption of glucose from the peritoneal cavity of mice has been determined by us on a former occasion. PROC. SOC. EXPER. BIOL. AND MED., 1925, xxiii, 122.

2940

The rate of absorption of a mixture of glucose and galactose.

CARL F. CORI.

[*From the State Institute for the Study of Malignant Disease, Buffalo, N. Y.*]

Previous work[1] has shown that each sugar has its own characteristic rate of absorption from the intestine and that this rate remains constant. Glucose was found to be absorbed at a rate of 0.178 gm. per 100 gm. of body weight per hour, galactose at a rate of 0.196 gm. per 100 gm. of body weight per hour. It seemed of interest to study at what rates glucose and galactose would be absorbed if a mixture of these two sugars was fed.

Experimental.

The method for the quantitative study of intestinal absorption has been described on a previous occasion.[1] A 60 per cent sugar solution, consisting of equal parts of glucose and galactose, was fed. The concentration of the two sugars in the same solution was determined in the following way. First the total sugar content of the solution was found. Glucose was then removed by fermentation with yeast. The sugar remaining in the fermented solution corresponded to the amount of galactose present. The amount of glucose present was calculated by difference.

TABLE I.

The rate of absorption of a mixture of equal parts of glucose and galactose. The rats were killed 2 hours after the sugar feeding.

Body weight.	Blood sugar.	Absorption coefficient* for glucose.	Absorption coefficient* for galactose.	Total absorption* coefficient
gm.	per cent.	gm.	gm.	gm.
137.8	0.217	0.118	0.086	0.204
145.3	0.200	0.103	0.065	0.168
155.4	0.246	0.109	0.079	0.188
160.5	0.213	0.108	0.062	0.170
121.7	0.221	0.101	0.078	0.179
136.9	0.242	0.097	0.070	0.167
117.0	0.170	0.122	0.077	0.199
Average:	0.215	0.108	0.074	0.182

*The absorption coefficient is the amount of sugar absorbed per 100 gm. of body weight per hour.

[1] *J. Biol. Chem.*, 1925, lxvi, 691.

It will be seen from Table I that the rate of absorption of glucose and galactose is considerably reduced if both sugars are absorbed simultaneously. We may speak of a mutual inhibition. The absorption of glucose taking place simultaneously with the absorption of galactose, inhibits the rate of absorption of the latter sugar and *vice versa.* If such an inhibition did not exist, the organisms would be flooded with sugar, whenever a mixture of two or more sugars is fed. If glucose alone is fed 0.178 gm. are absorbed per 100 gm. per hour; if galactose alone is fed 0.196 gm. are absorbed per 100 gm. per hour. This would make a total amount of 0.374 gm. sugar, if the same rate would prevail during the simultaneous absorption of these two sugars. However, Table I shows that the total amount of sugar absorbed is only 0.182 gm.

It is very striking that glucose is absorbed faster from the mixture than galactose, since the opposite is true if each of these two sugars is fed separately. In the case of the absorption of the mixture, if the rate of absorption of glucose is taken as 100, the ratio glucose to galactose is of the order 100:68.5. In the case of the separate absorption the ratio is of the order 100:110.

Summary.

When glucose and galactose are absorbed from a mixture of equal parts of these two sugars, the rate of absorption of both sugars is reduced to such an extent, that the total amount of sugar absorbed is not greater than if glucose alone or galactose alone were being absorbed.

2941

Toxin production of the streptococcus erysipelatis.

KONRAD E. BIRKHAUG.

[*From the Department of Bacteriology, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.*]

The toxins employed in these studies were prepared in Douglas' tryptic medium inoculated with cultures of *Streptococcus erysipelatis,* which were isolated from the erysipelatous lesions of patients ill with erysipelas. Among thirty-four strains grown at

37° C. for periods varying from six to ninety-six hours, the maximum toxin production was obtained in lots incubated for about 48 hours. Each of the 34 strains studied were found to yield uniformly toxic filtrates. A skin test dose of 0.1 cc. of a 1:1000 dilution of erysipelas toxic filtrate produced in the skin of susceptible persons a lesion, similar in nature to that obtained in the Schick and Dick tests, which measured more than 1.5 cm. in diameter. Complete neutralization of one skin test dose of the erysipelas toxin was obtained by mixing it with an equal amount of convalescent erysipelas serum, or with 0.001 cc. of erysipelas streptococcic rabbit or donkey sera. Neutralization of the erysipelas streptococcic toxin was not accomplished by Dochez' scarlatinal antistreptococcic serum, nor by normal rabbit or donkey sera. During the acute stages of erysipelas the patient's blood serum and urine contained a toxic substance which was completely neutralized by convalescent erysipelas serum and which disappeared from the patient's blood serum and urine as soon as twelve hours after the administration intramuscularly of 25 to 100 cc. of erysipelas antistreptococcic rabbit or donkey sera. If the disease persisted unchecked by the serum therapy, the skin reaction remained positive until defervescence and definite regression of the erysipelatous lesion occurred.

Positive skin reactions were obtained by one skin test dose of erysipelas streptococcic toxin in 27 per cent of apparently normal adults and in 21 per cent of normal school children. Among 19 persons with definite histories of single and recurrent attacks of erysipelas, 4 persons gave positive reactions with one skin test dose of the erysipelas streptococcic toxin. These findings add further evidence to our previous reports that a specific relationship exists between *Streptococcus erysipelatis* and erysipelas.

Minnesota Branch

University of Minnesota, December 2, 1925.

2942

Histological changes in the adrenal glands of guinea pigs subjected to scurvy and severe inanition.

BLANCHE LINDSAY and GRACE MEDES. (Introduced by J. F. McClendon).

[*From the Department of Physiology, University of Minnesota, Minneapolis, Minn.*]

McCarrison[1] described histological changes in the adrenal glands of guinea pigs fed a diet lacking in vitamin C. He reported hemorrhagic infiltration and degenerative changes in the cells of the cortex and medulla. The hemorrhagic areas are described as varying in size, situated in the cortex of the gland, and are circumscribed in character. The cells of the cortex are described as losing their tessellated appearance and manifesting a state of degeneration, which includes vacuolation and loss of the staining reaction of a portion of the nuclei.

Findlay[2] observed congestion in both cortex and medulla, but could not corroborate McCarrison's findings in regard to other cellular changes. Hemorrhage was observed only a few times.

Höjer[3] did not observe hemorrhage. The most pronounced change was simple atrophy, at first in connection with hyperemia.

Twelve guinea pigs were fed a scorbutic diet of alfalfa meal and wheat flour, equal parts by weight, oats *ad libitum* and powdered whole milk (Klim) corresponding to 50 cc. fresh milk

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[1] McCarrison, R., *Studies in Deficiency Disease.* London, 1921.

[2] Findlay, G. M., The Blood and Blood-vessels in Guinea Pig Scurvy, *J. Path. and Bact.*, 1921, xxiv, 446.

[3] Höjer, J. Axel, Studies in Scurvy, *Acta Paediatrica (Supplementum)*, 1924, iii.

daily. Others were subjected to severe inanition by receiving limited amounts of the above diet with 2 cc. orange juice added daily.

Histological observations upon the adrenal glands of the different animals subjected to scurvy and starvation reveal similar changes in the adrenals. These glands present varying degrees of the changes reported by McCarrison.[1] In all cases where a change has taken place, evidence of hemorrhagic infiltration is more pronounced than degeneration of the cells. The infiltration assumes a circumscribed appearance around the medulla, occurring between the columns of cells of the cortex along the connective tissue septa.

2943

Germinal epithelium of guinea pigs during early stages of scurvy.

GRACE MEDES. (Introduced by J. F. McClendon).

[*From the Department of Physiology, University of Minnesota, Minneapolis, Minn.*]

Guinea pigs were fed a scorbutic diet of alfalfa meal and wheat flour, equal parts by weight, oats *ad libitum,* and powdered whole milk (Klim) corresponding to 50 cc. fresh milk daily. The increase in weight for about 10 to 15 days was approximately at the same rate as control rats which received, in addition, 2 cc. of orange juice daily. They showed no symptoms of scurvy. At this time, however, histological changes characteristic of scurvy have already set in.

Observations made on the testes of guinea pigs, fed on a scorbutic diet and killed after ten days, show engorgement of the blood vessels with degeneration of seminal epithelium in some of the tubules. Cells in early stages of spermatogenesis were especially affected. Other tubules were normal and contained all stages of developing spermatozoa.

Guinea pigs kept for 30 to 40 days in a state of severe chronic scurvy show almost complete recovery of germinal epithelium after 17 days on an antiscorbutic diet.

2944

Cutaneous reaction to pneumococcic filtrates.

By W. P. LARSON.

[*From the Department of Bacteriology and Immunology, University of Minnesota, Minneapolis, Minnesota.*]

Pneumococcus filtrates supplied by Olsen have been injected into mice intraperitoneally. A limited number of mice which received one injection of the filtrate in this way, when killed 24 to 48 hours later, showed marked congestion of the lungs. Microscopic sections showed congestion with hemorrhage throughout the lungs.

The filtrate was injected intracutaneously in 17 normal individuals, as well as three convalescent pneumonia cases who had received pneumonia antitoxin. Nine of the 17 healthy individuals gave a positive skin test and 8 a negative reaction. The convalescent pneumonia patients were all negative. The positive skin tests were present in about 8 hours following the injection, reached the height of their reactions in about 24 hours, after which they began to fade. At the end of 48 hours the reactions had all disappeared.

These observations, considered with those presented by Olson, indicate that his filtrates contain a soluble toxin.

2945

Studies on pneumococcus filtrates.

J. G. OLSON. (Introduced by W. P. Larson).

[*From the Eli Lilly Research Laboratories, Indianapolis, Ind.*]

Following the work of W. P. Larson[1] on the production of an antipneumococcus serum by means of whole pneumococcus cultures treated with Sodium Ricinoleate, studies were made to determine the mode of action of the serum. Used clinically, Larson's serum promptly and strikingly relieves the symptoms of intoxication in a large percentage of cases of pneumonia; and inasmuch as the serum does not appear to possess a high titre of

[1] PROC. SOC. EXP. BIOL. AND MED., 1925, xxii, 172.

animal protective bodies, as ordinarily measured, other avenues of approach were tried.

It developed that we could produce the clinical and pathological symptomatology of pneumonia in suitable animals with certain sterile pneumococcus filtrates intraperitoneally, and they may thereby be caused to develop dyspnea, prostration, and even death. At autopsy the lungs show congestion, infiltration, hemorrhage and even hepatization.

Such filtrates, when injected into the skin of certain animals, *e. g.,* rabbits, produce a reaction, in 16 to 24 hours, for an area of redness one to several centimeters in diameter may develop, such a reaction slowly fading over a period of several days. By suitable dilution, it has been found possible to so control the dosage, that 0.1 cc. will cause an area of redness 1.0 cm. to 1.5 cm. in diameter to appear, and reaches a maximum in 16 to 24 hours.

The phenomena described may be successfully produced in other animals than those mentioned.

It is obvious, of course, that a restricted line of procedure must be followed in the preparation of the filtrates.

We have found that sera prepared by Larson's method possess, in high degree, power to prevent both the lung changes mentioned, and also the cutaneous reactions. Moreover, sera from certain animals that have been given one or more injections of such filtrates are found to possess power to prevent the reactions. There seems to be a definite quantitative relationship between the ability of a serum to inhibit the lung phenomena and to prevent cutaneous reactions. What is especially interesting is the fact that it is also apparently possible to measure the relative therapeutic power of such sera on the basis of their ability to inhibit the cutaneous or pulmonary changes, both in experimental animals and in clinical cases of pneumonia.

From the above experiments it would appear that a toxin specific to the pneumococcus has been demonstrated and that the action of the Larson serum and of the serum produced by the injection of the toxin is antitoxic. Indications are that the toxin may be of value in the production of active immunity to pneumococcus infections and may be utilized as a measure of resistance.

Exhaustive experiments designed to throw light on the nature and mode of action of the toxin and antitoxin are being carried out.

Pacific Coast Branch

Lane Hospital, Stanford University Medical School, December 9, 1925.

2946

The distribution of water between serum and corpuscles in experimental anemia.

MEYER BODANSKY and O. G. DRESSLER. (Introduced by R. E. Swain).

[*From the Department of Chemistry, Stanford University, Stanford University, Calif.*]

It has been shown previously[1] that in experimental anemia due to acetylphenylhydrazine, the volume of the individual red corpuscle is increased, this being usually accompanied by a disproportionate increase of the unsaturated fatty acid content of the corpuscles. In the present work, we have been especially interested in determining possible changes in the water equilibrium between serum and corpuscles in anemia. We have found that the water content of the serum of normal dogs is about 92.0 per cent and of normal corpuscles about 65.0 per cent. In the anemic condition, there is an increase in the water content of the serum, which, however, is very slight, usually less than 1.0 per cent. On the other hand, in the corpuscles the water increase is much greater. Values of about 5.0 per cent above the normal are frequent. The following data are typical:

Dog No.	Date 1925	Red count in millions	Corpuscle vol. $V \times 10^{-8}$ cm.	Hemoglobin in corpuscle $Hb \times 10^{-8}$ mg.	Per cent water by weight.	
					Cells	Serum
			V	Hb.		
3	Oct. 14	7.76	5.35	1.74	65.25	91.64
	Oct. 22	2.22	9.01	2.46	70.07	92.26
6	Oct. 20	8.64	5.45	2.11	64.90	91.46
	Oct. 23	4.40	7.27	1.74	69.30	91.79
10	Oct. 29	8.19	5.07	2.00	64.70	92.30
	Nov. 2	3.46	5.94	1.49	69.50	92.50

[1] Bodansky, M., *J. Biol. Chem.*, 1925, lxiii, 239.

2947

Emetic dose of digitalis in pigeons as an index of the therapeutic dose in man.

P. J. HANZLIK and H. A. SHOEMAKER. (University of Oklahoma).

[*From the Department of Pharmacology, Stanford University School of Medicine, San Francisco, Calif.*]

Emesis, as an index of digitaloid action, occurs promptly and characteristically after the intravenous administration of the digitaloid preparations in pigeons whose vomiting mechanism appears to be rather sensitive. The technique of determining the emetic dose is simple.

The digitaloid preparation is injected from an accurately graduated Luer (tuberculin) syringe into the wing vein of a pigeon of about 300 gm. body weight, held conveniently by an assistant. Then the pigeon is at once replaced into a cage for observation of vomiting, which is recognized by downward craning movements of the head preceded, sometimes by salivation and lachrymation and accompanied by ruffling of neck feathers, and, usually, flapping of the wings with occasional expulsion of gravel. Several such vomitings occur at the end of 5 to 10 minutes, depending on the dosage of the preparation used. Since the pigeons recover completely at the end of 2 to 3 days, they may be used over again for confirmation, and apparently as long as the wing veins permit further injections. The digitaloid preparations do not require previous evaporation, as in the case of most bioassay methods; but sodium chloride in isotonic concentration should be added to infusions, and tinctures should be diluted with about an equal part of 0.85 per cent sodium chloride before injection.

With this method the minimal emetic dose (M. E. D.) of a good tincture of digitalis (U. S. P.) is about the same as the full therapeutic or "minor toxicity" dose determined by Eggleston[1] for man, namely, 0.3 cc. (30 mg. digitalis) per kilo of body weight, or about 15 cc. for a 50 kilo man. Hence, it seems that the method should be valuable in determining the probable full therapeutic dose since emesis is one of the earliest signs of digitalis action in man; or, at least, it should be a valuable supple-

[1] Eggleston, *Arch. Int. Med.*, 1915, xvi, 1.

mentary test to bioassays depending on fatal dosage. The M. E. D. of ouabain is about 0.045 mg. per kilo.

Use of this method in bioassaying different tinctures and infusions of digitalis has given results, as good as, and better than the one-hour frog method. It has the following advantages over the official (U. S. P.) frog method, the cat method, and in fact, most bioassays; no operation or anesthesia is required, uniformity, simplicity, convenience, economy of time and material, ease of application, the index is about therapeutic, while all other methods depend on fatal dosage and the accuracy appears to be equal to that of any method. Provisionally it appears that standardization of pigeons will not be necessary. However, the influence of various factors and further details and possibilities of the method are being investigated.

2948

The occurrence of cysts of Councilmania lafleuri Kofoid and Swezy in the duodenal drainage.

C. A. KOFOID.

[*From the Department of Zoology, University of California, Berkeley, Calif.*]

A case of infection by *Endamoeba dysenteriæ* and *Councilmania lafleuri,* amœbae of the human digestive tract, was treated in May, 1921, for the former, by the emetin-bismuth-iodide method. The results were negative for it on eight stool examinations six to seven months later. Duodenal drainage from this case, examined after treatment, contained a considerable number of cysts of *C. lafleuri* of the characteristic size, shape, number of nuclei, and nuclear structure. There were also some empty cysts, bile-stained, of similar appearance in the fresh drainage.

Duodenal drainage was also examined from a second case of infection by *Councilmania lafleuri* but no *E. dysenteriæ.* In this case 67 stool examinations have been made since November 23, 1922, with 41 positives. In the heavy bile of the first bottle of duodenal drainages a number of typical cysts of *C. lafleuri* have been found on two tests of four made. Upon staining by iron

hæmatoxylin these cysts had both the cytoplasmic and nuclear structure of the cysts of this parasitic amœba discharged in the stool.

2949

On the culture in vitro of Councilmania lafleuri and Endamoeba coli.

CHARLES A. KOFOID and ENA A. ALLEN.

[*From the Department of Zoology, University of California, Berkeley, Calif.*]

During the past year cultures have been made by us from fifty stools from eight cases of infection by intestinal amœbae. Three of these had *Endamoeba coli* and *Councilmania lafleuri,* three had *C. lafleuri,* and two had *E. coli.* Upon culture of stools from the first group both species of amœba appeared in the cultures from two of the three cases. Of the third only one attempt with 6 tubes was made. Not infrequently attempts at culture on any case will fail from some stools. In the second group containing infections of *C. lafleuri,* this species only was obtained in cultures. In the third group containing *E. coli* this was obtained in culture from one of the two cases. One stool only, however, was used from each case.

Cultures have been made from 49 stools. All cases cultured had infections of *Blastocystis.* This organism invariably overgrows cultures of amœba sooner or later, generally within four to six days in Boeck's media. Transplants of *C. lafleuri* must be made every twenty-four to forty-eight hours. If, however, the dextrose is omitted, cultures have survived with transplantation for fourteen days, and in one instance only motile amœbae survived in the same tube for twelve days. In Locke's solution with 0.5 per cent defibrinated rat's blood plus 1:60500 acroflavine transferred cultures were continued for twenty-one and twenty-eight days in two instances. In case of *E. coli* no culture could be continued beyond seven days in any of the media. It is much more susceptible than *C. lafleuri* and is always much less abundant than that species in both the stools and the cultures.

In a peptone medium made from Lilly's liquid peptone reduced one half by evaporation, and removal of the alcohol, plus 0.5 per cent sodium chloride, with and without egg slant, only *C. lafleuri* grew, though *E. coli* was also present in the stool.

On the electric warm stage, cultures will show *E. coli* and *C. lafleuri,* in motile activity, occasionally in the same field. The differences between the two in appearance and behavior in this medium are evident and are identical in their main features with those of the same amœbae in motile phases from warm stools. *E. coli* has a sluggish behavior, rolling along leisurely. Clear pseudopodia have never been seen by us in *E. coli.* As it progresses pseudopodial extensions are immediately granular in structure. They are also somewhat conical in contour. *C. lafleuri* on the other hand moves rapidly when in locomotion. Its pseudopodia are thrown out with explosive suddenness and are invariably extremely clear and hyaline. Their contour is broadly rounded, varying with the degree of acidity. The pseudopodia gel quickly and often persist unchanged for a brief time. Granular pseudopodia have never been seen by us in *C. lafleuri.*

The color of the two amœbae *in vivo* is characteristically different. *E. coli* has a bluish gray tinge and a more finely granular structure. *C. lafleuri* is pale yellowish green and more coarsely alveolar.

The *E. coli* with clear pseudopodia which Thomson and Robertson[1] recovered in culture from stools is undoubtedly not *E. coli.* It may be *C. lafleuri.*

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[1] Thomson, J. G., and Robertson, Andrew, *J. Trop. Med. Hyg.,* 1925, xxviii, 345-349.

2950

The structure of starch grains from wheat grown under constant conditions.

H. L. VAN DE SANDE-BAKHUYZEN. (Introduced by C. L. Alsberg).

[*From the Food Research Institute and the Department of Botany of Stanford University, Calif.*]

This is a report upon the starch of the seed of Hard Federation wheat grown under continuous constant illumination by Mazda C lamps, the other external conditions such as temperature, humidity, water- and salt-content of the sand in the pots being kept as constant as possible.[1]

The starchgrains did not show the lamellation or rings that can be seen in the starch of ordinary wheat seeds. This difference is all the more striking if the two kinds of starch are compared, after heating to a temperature at which the grains begin to swell, while their border is still refractive. Inside the swollen unlamellated grains, very refractive radial needles can be seen, attached to the refractive border of the grain or to the circumference itself, while the rest of the grain may be quite translucent. On the upper and lower sides of the surface of the grains, the needles are seen in optical section as refractive globules; in the optical plane, between the upper and lower surface, they are seen at their full length. They are tapered towards the center, resembling pyramids with a base of 2-3μ. The lamellated starchgrains in the ordinary seeds also show needles after they have been heated to this temperature. These needles extend to the first distinct non-refractive ring, and are much shorter absolutely and relatively. The needles in the ringless starch sometimes have a length of 17 to 20μ, which is about 45 per cent of the total diameter of the grain; in the field-grown seeds they are no larger than 5μ, or about 10 per cent of the diameter. Ten to twenty or more rings can be counted.

A suspension of ringless starch in water heated over a flame applied to the margin of the slide shows all graduations of swelling. In this way it was easy to follow the effect of different

[1] The observations upon growth and metabolism under these conditions will be presented later.

temperatures by studying the different stages of swelling. The unheated grains show some needles which are visible through the refractive border of the grain; these are the needles which are discussed above. They are the thickest and the most resistant to higher temperatures. If the temperature is raised, the refractive border of the grain loses its refractivity, so that the interior of the grain, *i. e.*, the needles can be more easily observed. There are both thick and thin needles, filling the interior of the starch-grain, except at the hilum. With increase in temperature, the thinner ones are the first to dissolve, so that only the thicker ones remain. These needles are not radial cracks. The bases of the thinner needles, which are their thickest parts, are still visible. The narrow, tapered ends of the thicker needles also begin to dissolve, so that the thicker needles become shorter as well. Finally, they lose their refractivity and the starchgrains become large, swollen sacks without much structure. These stages were followed throughout the heating and cooling process.

These operations leave no doubt that these ringless or unlamellated starchgrains are composed of masses of radially arranged refractive needles, thinner towards the center, thicker towards the circumference. At the circumference their bases are fused to form a very refractive border. This border obscures the radial structure of the unheated starch, so that only the thickest needles are visible.

The occurrence of large needles must be correlated with the absence of rings. This is also borne out by the following observation. If starch from field-grown grains is heated to the swelling temperature, the rings then consist of small particles, as pointed out in a previous paper.[2] Now, particles in successive rings may often be seen oriented in a radial direction, suggesting that needles had fallen apart on heating. The parts of the needles which correspond to the non-refractive rings have been dissolved by heat. The parts lying within the refractive ring, however, remain. Moreover, in ordinary unheated starch large needles can also be seen.

Careful examination under magnifications of 1300 to 1600 diameters shows that even in the ringless starch there is, in many grains, slight indication of the presence of one or two rings, which, however, are very indistinct. They cannot be followed all

[2] PROC. SOC. EXP. BIOL. AND MED., 1925, xxiii, 195.

around the grain. It is very probable that these indistinct rings are due to fluctuations in external conditions. During ripening of the seeds, illumination was twice interrupted accidentally for periods of 2 and 2½ hours. The temperature decreased during that time 7° C. and 5° C. respectively. Some of the needles are interrupted by one of these very faint rings, so that they fall apart into two or three shorter portions. Some needles overlap such a ring and only a refractive spot can be seen at the place which corresponds to the ring.

Whether or not these needles are crystals will not be discussed here, but it is evident that Meyer is right when he states that the starch grains have a "radialtrichitische Struktur". In the ringless starch, the needles are nearly homogenous and probably all of them stretch from the border to the hilum in unheated starch. The starch grown under field conditions is composed of the same radially arranged needles, but these have a different refractivity at the places corresponding to the less refractive and more refractive rings.

As was discussed in the previous paper[2] the more and less refractive rings of the starchgrains have to be considered as composed of more and of less dehydrated amylose. The phenomena caused by heating to the swelling or gelatinization temperature consist of hydration. Partially dehydrated amylose loses its refractivity or goes into solution, according to the degree of its previous degree of dehydration. The thicker needles offer more resistance to hydration than the thinner ones. After cooling, dissolved amylose is again partially dehydrated.

If at a certain stage of growth of a starchgrain n needles cover 1 cm.^{2} of the inner surface of the grain, $4n$ needles have to develop per cm.^{2} if the radius of the starchgrain increases 100 per cent, or else the diameter of each needle has to increase 100 per cent. As the needles are actually wider at their bases, this phenomenon can only be explained on the assumption that new starch substance is added to the base of the needles.

The formation of rings of different refractivity has to be ascribed to different stages of hydration of the amylose during or after its apposition. The hydration of the amylose will depend on the hydration of the protoplast, *i. e.*, on the amount of turgescence of the cell. This depends (other conditions remaining constant) largely on the transpiration. It is, therefore, probable that

not only the periodicity of the illumination will determine the periodicity of the dehydration of the amylose, but also to a large extent the periodicity of the transpiration. The transpiration factor has not been controlled by previous investigators.

2951

Alcohol and the sex ratio in mice.

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An animal heterozygous for a character produces two kinds of germ cells with respect to that character. Since these two classes of germ cells differ in their genetic potentialities, it is conceivable that they may also differ in their ability to react to varied environmental conditions. Critical tests of this question are difficult to devise, one of the best thus far being that introduced by Stockard[1] in his alcohol inhalation experiments. By use of Stockard's method evidence has been obtained which indicates that when alcohol is thus introduced into the tissue of the fowl, germ cells are differentially affected according to their general vigor,[2] as well as on the basis of some of their genetic differences.[3] In the mouse, in which the male is presumably heterozygous for the sex chromosomes, Bluhm[4] found a much higher sex ratio after administering alcohol to the male parent by subcutaneous injections. The difference was attributed to a differential effect on the two classes of sperm cells. Bluhm's work has been questioned[5, 6] because of the low sex ratio in the controls (80 males: 100 females in a total of 965 young, as compared with a ratio of 122:100 among those sired by alcohol-injected fathers). The

1 Stockard, C. R., *Amer. Nat.*, 1913, xlvii, 641.
2 Pearl, Raymond, *J. Exp. Zool.*, 1917, xxii, 125.
3 Danforth, C. H., *J. Exp. Zool.*, 1919, xxviii, 385.
4 Bluhm, Agnes, *Sitz. Ber. d. Preuss. Akad d. Wiss.*, 1921, xxxiv, 549.
5 Pearl, Raymond, *Eug. Rev.*, 1924, xvi, 1.
6 Hanson, Frank Blair, and Heys, Florence, *Genetics*, 1925, x, 351.

more recent data of Parkes[7] shows a wide seasonal variation in the sex ratio of mice which may help to explain the apparent discrepancy in Bluhm's results.

The present report covers three short experiments in which male mice were treated with alcohol fumes in the usual way. Cylindrical glass specimen jars of about 20 cm. diameter and 5.5 liters capacity were fitted with false bottoms of perforated paraffined wood supported on short metal legs. From 75 cc. to 100 cc. of 95 per cent ethel alcohol were poured in the jar, the false bottom inserted and the cover put in place. After some minutes, when the atmosphere had become saturated with alcohol vapor, a mouse was slipped in as quickly as possible and the cover replaced. Treatments lasted for at least an hour, except when an individual gave evidence of extreme prostration. With rare exceptions, two treatments were administered every day. In each experiment three males were used and they were allowed to begin breeding about a week after the treatments were commenced.

In the first experiment, (August, 1924), pregnant females were sacrificed during the last days of gestation so that only foetuses of 18 to 20 days were considered. This experiment had to be abandoned after 53 foetuses had been examined. Among these 34 were males and 19 females, giving a sex ratio of 178.9 males: 100 females. There were no control data, but judging from MacDowell and Lord's records[8] (2525 young) the normal ratio for this age is probably from 100 to 103.4. Even though these numbers are small they seem indicative of an effect produced by alcohol treatment.

In the second and third experiments the young were born but, both in the experiments and the controls, they were immediately killed and their sex determined by dissection. The results are summarized in the following table:

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[7] Parkes, A. S., *Brit. J. Exp. Biol.*, 1924, i, 223.

[8] MacDowell, E. Carleton, and Lord, Elizabeth M., PROC. SOC. EXP. BIOL. AND MED., 1925, xxii, 389.

	Second Experiment.			Third Experiment.			Both Experiments Combined.			
	Date	Number	Ratio	Date	Number	Ratio	Total	Males	Females	Ratio
Males treated	Jan. 17 to Mar. 14	119	153.1	May 22 to Sept. 29	225	117.9	374	210	164	128.0
Males not treated	Jan. 5 to Feb. 28	715	104.8	May 15 to Aug. 17	417	100.4	1132	575	557	103.2

In the second experiment the percentage of males is 61.34 ±3.03 among the young of alcoholized fathers, and 51.18 ±1.26

among those from untreated males. The difference is a little more than three times its probable error and might therefore be regarded as statistically significant. In the third experiment the difference is slightly less than three times its probable error (2.7) but still clearly marked. When the three experiments are combined, using MacDowell and Lord's figures as well as our own as control for the first series, the difference between the number of males produced in the experiments and in the controls is about 3.3 times its probable error. If the sex ratio of the mouse was clearly stable there would be little doubt that this mode of treatment does have a definite result in raising the proportion of males, but since the ratio is variable as shown by Parkes, it is obvious that more data should be obtained from different strains and at different seasons. It is also clear that any further experiments in this direction should provide for the best possible controls as regards strain, age, and breeding histories of both male and female parents. As the question now stands, all the available data indicate that in the mouse alcoholization of the male parent results in a slight rise of the sex ratio.

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A non-polarizable micro-electrode. Preliminary report.

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The ability to penetrate a single living cell with very little injury to it by micro-needles and micro-pipettes has proven to be very fruitful in the relatively short time since this technique has been developed by Barber, Chambers, Taylor, and Pèterfi. To be able to penetrate into the interior of a single living cell by means of electrodes that are minute enough and at the same time functional, so that direct and accurate electrical measurements can be made of the electrical conditions attending the stimulation and the normal functioning of a Protisten cell, is obviously of considerable experimental significance.

A micro-electrode of that nature that is non-polarizable has been perfected and tried out by the writer with satisfactory results. Small quartz glass pipettes (about 0.5 mm. in diameter) are drawn out over an oxygen flame to minute points with openings of about 1-2 microns. These pipettes are filled with dialyzed and filtered agar that has been impregnated with M/10 KCl. The agar is dialyzed electrically for the removal of inorganic impurities. The current (D. C.) is sent in both directions for equal periods of time to remove both the anions and cations. It may be stated that the dialyzed agar even after impregnation with KCl, at room temperature is in liquid form (like milk) due probably to the loss of water by the agar because of the complete removal of some anion that is necessary for gelation. The pipettes are sealed into a glass tube (pipette shank) that is filled with the same agar and in which is immersed from the opposite opening a C. P. silver wire coil that has been coated with AgCl by electrolysis. The entire system is made air tight with dental cement and is then suited for mounting on a micro-manipulator. Because of the minuteness of the pipette point, the resistance of the electrodes is very great, but with a sensitive galvanometer extremely small differences of potential can be measured.

The writer began to work on the perfection of a non-polarizable micro-electrode about a year ago with Dr. C. V. Taylor. The use of AgCl and agar impregnated with KCl for a non-polarizable system was originally suggested by him, and although we succeeded in making up a pair of electrodes they proved to be too unstable to function. In the detailed account of the technique that is to follow shortly, it will be seen that the work was done with extreme accuracy, and that all precautions were taken to make the electrodes stable and give comparable results. The writer has demonstrated that with one of the most sensitive galvanometers no potential difference between the two electrodes thus constructed can be detected.

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The rôle of water in the starch iodine reaction.

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A number of investigators[1, 2, 3] have reported that "dry" starch will not react with iodine to produce the well-known blue substance, starch iodide.

Starch is a very hygroscopic material,[4, 5] and like other hygroscopic substances, when in contact with the atmosphere, will tend to absorb water until the vapor pressure of the absorbed water is in equilibrium with the vapor pressure of the surrounding air.[6] Many of the researches involving "dry" starch were carried out with starch in more or less complete moisture equilibrium with the atmosphere, hence by no means really dry.

The conditions of preparation, storage, and other treatment which affect moisture content, of the starch used should be fully described in work of this sort.

Since the literature indicates that starch at a certain degree of dryness will no longer yield blue starch iodide when treated with iodine, it seemed worth-while to determine approximately the water concentration necessary that this reaction may take place.

In the following investigation the starches used were prepared according to the method of Alsberg and Rask[7] and ground after the method of Alsberg aand Perry,[8] and then stored for varying periods of time in reagent bottles at room temperatures. Such preparations were of course in moisture equilibrium with the air in the containing vessel.

In ground starch the comparatively dense outer layer of the starch grain, which as Denniston[9] pointed out, reacts more slowly

1 Tomlinson, C., *Phil. Mag.*, 1885, xx, 168.
2 Stocks, *Chem. News*, 1887, lvi 212; 1888, lvii, 183.
3 Andrews and Goettsch, *J. Am. Chem. Soc.*, 1902, xxiv, 865.
4 Archbold, *J. Soc. Chem. Ind.*, 1887, vi, 83.
5 Reichert, Carnegie Inst. Wash. Pub. No. 173, Pt. 1, 1913, p. 167.
6 Fisher, E. A., *Proc. Roy. Soc. A.*, 1923, ciii, 139.
7 Alsberg and Rask, *Cereal Chem.*, 1924, i, 7.
8 Alsberg and Perry, PROC. SOC. EXP. BIOL. AND MED., 1924, xxii, 60.
9 Denniston, *Proc. Wis. Acad. Sci.*, 1907, xv, 664.

with iodine than the inner portion of the grain, is cracked and chipped, and are more favorable for rapid formation of starch iodide than when the intact grain is used.

The technique employed was essentially that of Gobley.[1]

Samples of ground wheat and potato starches were placed on separate glass slides and spread out in a thin layer with a spatula. These were then placed in a small, empty dessicator. Another slide, containing more than enough iodine crystals to saturate the dessicator with iodine vapor was also placed in it, and the dessicator was closed and allowed to stand 24 hours at room temperature.

On examination after 24 hours neither starch showed a trace of blue color. Both were brown, due to condensed and probably some adsorbed iodine.

Above experiments were repeated. After 24 hours the results noted above having recurred, water was added to the bottom of the dessicator in more than sufficient amount to saturate the system. Neither the starch nor the iodine were in contact with liquid water. The system was closed and allowed to stand. In less than half an hour traces of blue appeared on the starches, and on examination 24 hours later both starches were deep blue in color. The starch particles at this time adhered to one another, but when any mass was broken up it was observed that the blue color extended homogeneously throughout.

Starches and iodine were arranged in a dessicator as above, but enough water was added to the system to saturate it at the same time that the starches and iodine were put in. In a short time traces of blue were apparent, and after 24 hours both starches showed a deep blue-black color. It should be noted that there seemed to be no difference in final shade between potato and wheat starch iodide.[5]

Samples of wheat and potato starches were added as before to a system already saturated with both water and iodine vapor. The results differed only in a more rapid development of blue color.

All the above experiments were repeated using the same starch preparations as above, but first exposing the samples taken directly to the laboratory air for 24 hours. This procedure did not cause any difference in results.

The experiments show that there is a threshold concentration of moisture requisite for the starch iodine reaction. Furthermore, since the moisture content of the starch is a function of

the environmental free water concentration, this latter is the controlling factor in so far as the aqueous features of the reaction are concerned. It has been known for many years that there is a threshold concentration of iodine necessary for the reaction.[10]

The value of this threshold water concentration lies between that producing saturation pressure at approximately 22 degrees Centigrade, and above the water concentration present usually in the laboratory air of the Department of Chemistry at Stanford University during the clear season, when the humidity averages below 50 per cent.

At the threshold concentration of iodine, water being in excess, the formation of starch iodide as measured by the development of the blue color, is very rapid. At the threshold concentration of water, iodine being in excess, the reaction seems to be comparatively slow. However, it may be that other factors are concerned in the latter case.

[10] Meineke, *Chem. Ztg.*, 1894, xviii, 157.